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Tetracycline Susceptibility Testing and Resistance Genes in Isolates of *Acinetobacter baumannii-Acinetobacter calcoaceticus* Complex from a U.S. Military Hospital[▽]

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Infections with multidrug-resistant *Acinetobacter baumannii-Acinetobacter calcoaceticus* complex bacteria complicate the care of U.S. military personnel and civilians worldwide. One hundred thirty-three isolates from 89 patients at our facility during 2006 and 2007 were tested by disk diffusion, Etest, and broth microdilution for susceptibility to tetracycline, doxycycline, minocycline, and tigecycline. Minocycline was the most active in vitro, with 90% of the isolates tested susceptible. Susceptibilities varied significantly with the testing method. The acquired tetracycline resistance genes *tetA*, *tetB*, and *tetA*(39) were present in the isolates.

Multidrug-resistant *Acinetobacter baumannii-Acinetobacter calcoaceticus* complex bacteria cause infections in traumatic wounds in U.S. military personnel injured in Iraq and Afghanistan (9). A previous study of isolates recovered at our facility during 2003 to 2005 found these isolates to be highly susceptible to minocycline (6), and our clinical experience with minocycline for the treatment of these infections has been favorable (5). The possibility of increasing resistance to minocycline is of concern, as it would further restrict therapeutic options for multidrug-resistant isolates and may necessitate the use of agents with a greater potential for toxicity, such as colistin. Tigecycline has been proposed as a therapeutic option for multidrug-resistant *A. baumannii-A. calcoaceticus* complex infections (Tygacil package insert, Wyeth Pharmaceuticals, 2005), but its in vitro activity against isolates of military origin is uncertain.

The antibiogram for tetracycline, doxycycline, minocycline, and tigecycline was determined by using 133 clinical isolates from blood and wound infections of 89 patients admitted to our facility in 2006 and 2007. Serial isolates were included only when separated by at least 72 h. Susceptibilities to each antimicrobial were determined from a single measurement by disk diffusion (Becton Dickinson, Sparks, MD), Etest (AB Biodisk, Solna, Sweden), and broth microdilution by the methods of the Clinical and Laboratory Standards Institute (CLSI) (3). Discordant results were tabulated as very major errors (reported susceptible when resistant), major errors (reported resistant when susceptible), or minor errors (reported intermediate when resistant or susceptible or vice versa). CLSI interpretive criteria were used (4), except for tigecycline, for which no interpretive criteria are available.

Clonal relationships were assessed by pulsed-field gel electrophoresis (PFGE) with ApaI digestion (Centers for Disease Control and Prevention PulseNet protocols; http://www.cdc.gov/PULSENET/protocols.htm [accessed 25 July 2007]), with modifications for *Acinetobacter*. Strains were considered to be related if their Dice coefficient was ≥85% (Bionumerics; Applied Maths Inc., Austin, TX). The 89 single-patient isolates

TABLE 1. Broth microdilution susceptibilities and MIC distribution from 89 single-patient *A. baumannii-A. calcoaceticus* complex isolates

Agent	MIC_{50}	MIC_{90}	% S[a]	No. of isolates for which MIC (μg/ml) was:								
				≤0.06	0.125	0.25	0.5	1	2	4	8	≥16
Tetracycline	≥16	≥16	10.1	0	0	0	0	1	4	4	4	76
Doxycycline	≥16	≥16	31.5	4	4	1	1	11	2	5	12	49
Minocycline	1	4	89.9	9	0	5	14	35	12	5	6	3
Tigecycline	4	8		0	1	3	2	8	29	9	30	7

[a] S, susceptible.

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TABLE 2. Rates of susceptibility testing errors among 133 clinical isolates by antimicrobial agent and testing method

Agent and testing method[a]	% Susceptible	Very major error[b] (%)	Major error[c] (%)	Minor error[d] (%)
Tetracycline				
DD	1.5	0	0	10.5
ET	3.8	0	0	9.0
BMD	9.0			
Doxycycline				
DD	22.6	0	5.3	13.5
ET	22.6	0	3.0	18.8
BMD	30.1			
Minocycline				
DD	36.8	0	1.5	53.4
ET	69.9	0	1.5	18.0
BMD	88.7			

[a] DD, disk diffusion; ET, Etest; BMD, broth microdilution.
[b] Very major error, susceptible by DD or ET when resistant by BMD.
[c] Major error, resistant by DD or ET when susceptible by BMD.
[d] Minor error, intermediate by DD or ET when susceptible or resistant by BMD or susceptible or resistant by DD or ET when intermediate by BMD.

were confirmed to be members of the *A. baumannii-A. calcoaceticus* complex by amplification of $bla_{\text{OXA-51-like}}$ β-lactamase genes and by amplified ribosomal DNA restriction analysis (ARDRA) with the restriction enzymes AluI, HinfI, HhaI, RsaI, MboI, and MspI (12, 13).

The presence of tetracycline resistance genes in 89 single-patient isolates was determined by PCR amplification for the *tetA*, *tetB*, *tetH*, *tetL*, *tetM*, *tetA*(39), and *tetA*(41) genes with previously published primer sequences by previously described methods (1, 8, 11). Data were analyzed according to susceptibility phenotype, PFGE type, and *Acinetobacter* species as determined by ARDRA.

Consistent with our clinical experience and prior laboratory observations, minocycline had the highest in vitro activity, reflected in the MICs for 50 and 90% of the strains tested (MIC_{50} and MIC_{90}, respectively) and the percentage of isolates susceptible according to the broth microdilution method (Table 1). The in vitro activities of tetracycline and doxycycline were low. The modal MIC of minocycline was more favorable than that of tigecycline.

The accuracy of the disk diffusion and Etest methods was evaluated by comparing the results of 133 isolates tested by these methods to the reference method of broth microdilution (Table 2). Minocycline susceptibility varied widely by testing method, from 36.8% by disk diffusion to 88.7% by broth microdilution. No very major errors were observed. Etest committed fewer major errors than disk diffusion for doxycycline, while the methods were equivalent for minocycline. Etest committed fewer minor errors than disk diffusion for tetracycline and minocycline. There was no predominating type of minor error for either testing method (data not shown).

The accuracy of disk diffusion testing for determining minocycline susceptibility was poor. By this method, 51% of the isolates in this study would have been incorrectly reported as nonsusceptible to minocycline. This likely occurred due to clustering of the inhibitory zone diameter at or near the susceptibility breakpoint for minocycline in many isolates. Utilizing the Etest would have incorrectly reported 18% of the isolates as nonsusceptible to minocycline, a considerable improvement over disk diffusion but still exceeding the allowable limits of the CLSI (2). High disk diffusion error rates for *Acinetobacter* have been previously reported for tetracycline (10) and tigecycline (7).

We observed 24 distinct PFGE types among the 89 single-patient isolates. Approximately two-thirds of the isolates were represented by four PFGE types (Table 3), composed of *A. baumannii*, *A. calcoaceticus*, or isolates that were indistinguishable between these by ARDRA (considered to be *A. baumannii-A. calcoaceticus* complex isolates). All isolates carried the $bla_{\text{OXA-51-like}}$ β-lactamase gene. Three PFGE types comprising 61% of the isolates were associated with a minocycline MIC_{90} within the susceptible range. Among these three PFGE types, the percentages of isolates susceptible to tetracycline, doxycycline, and minocycline were 0, 18.5, and 100%, respectively.

The tetracycline resistance determinants *tetH*, *tetL*, *tetM*, and *tetA*(41) were not found in any isolates. The frequencies of

TABLE 3. MIC_{90}s and tetracycline resistance gene distribution by phenotype, PFGE type, and species among 89 single-patient *A. baumannii-A. calcoaceticus* complex isolates

Phenotype, PFGE type, or species	*n*	MIC_{90} (μg/ml) of:				No. with resistance gene:		
		TET	DOX	MIN	TGC	*tetA*	*tetB*	*tetA*(39)
MIN-S + DOX-S + TET-S	9	4	0.5	0.25	1	0	0	0
MIN-S + DOX-S + TET-NS	19	≥16	4	1	4	4	0	1
MIN-S + DOX-NS + TET-NS	52	≥16	≥16	2	≥16	13	6	33
MIN-NS + DOX-NS + TET-NS	9	≥16	≥16	≥16	8	0	9	0
PFGE type 1 (*A. baumannii-A. calcoaceticus* complex)	34	≥16	≥16	2	8	0	1	32
PFGE type 2 (*A. baumannii*)	14	≥16	8	2	2	14	0	0
PFGE type 3 (*A. baumannii*)	6	≥16	1	0.5	4	0	0	0
PFGE type 4 (*A. calcoaceticus*)	7	≥16	≥16	8	8	0	7	0
All other PFGE types	28	≥16	≥16	≥16	8	3	7	2
A. baumannii	38	≥16	≥16	8	4	16	6	1
A. calcoaceticus	12	≥16	≥16	8	8	0	7	0
A. baumannii-A. calcoaceticus complex	39	≥16	≥16	2	≥16	1	2	33

[a] MIN, minocycline; DOX, doxycycline; TET, tetracycline; TGC, tigecycline; S, susceptible; NS, nonsusceptible.

tetA, *tetB*, and *tetA*(39) increased with advancing resistance phenotype but were less common in the most resistant phenotype. *tetA* was predominantly found in PFGE type 2 (*A. baumannii*). *tetB* was found in all species and was more common in the less frequent PFGE types. *tetA*(39) was abundant in PFGE type 1 (*A. baumannii-A. calcoaceticus* complex) isolates retaining susceptibility to minocycline. *tetB* was found in isolates resistant to all of the agents tested, as well as in isolates retaining susceptibility to minocycline.

Multidrug-resistant *A. baumannii-A. calcoaceticus* complex bacteria remain an important cause of infection around the world. Minocycline is active against isolates of military origin, even when susceptibility to other tetracyclines and tigecycline has been lost. Determining minocycline susceptibility is hampered by important differences in accuracy between susceptibility testing methods, with the disk diffusion method significantly underestimating the proportion of susceptible isolates. Thus, in cases of infection with multidrug-resistant *A. baumannii-A. calcoaceticus* complex bacteria where minocycline therapy is desirable, it may be prudent to perform broth microdilution testing to obtain the most accurate susceptibility determination.

The opinions or assertions contained herein are our private views and are not to be construed as official or reflecting the views of the Department of the Army, the Department of the Air Force, the Department of Defense, or the U.S. Government.

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